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PATENT

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In re Application of: Steidler et al.

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For: USE OF A CYTOKINE-PRODUCING LACTOCOCCUS STRAIN TO TREAT COLITIS

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Enclosed is a copy of Priority Document 98203529.7 filed 20 October 1998 for the above-referenced application.

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Use of a cytokine-producing *Lactococcus* strain to treat colitis.

The invention generally relates to an administraton strategy for the delivery at the intestinal mucosa of cytokines, preferably of acid sensitive anti-inflammatory agents, such as IL10 and/or a soluble TNF receptor via the oral route. The prefered feature according to the invention is the inoculation with a suspension of live recombinant *Lactococcus lactis* cells, which had been engineered to produce the respective proteins. As example, mice were used in which a chronic inflammation of the distal colon had been induced by administration with dextran sulfate sodium (DSS). The treatment as scored by histological evaluation clearly resulted in a regression of the inflammation and disease symptoms. The finding is highly unexpected since, in order to exert activity at the colon following oral administration, the delivery system needs to pass the acidic environment of the stomach and the upper part of the small intestine respectively.

The immune system in a mammal is diverse and complex and includes natural and adaptive immune mechanisms and reactions. The immune system is often described in terms of either humoral or cellular immune responses. Humoral immunity refers broadly to antibody production and actions by B-cells; cellular immunity is mediated by cells including T-cells, dendritic cells, neutrophiles, monocytes and macrophages. T-cells and B-cells are two categories of lymphocytes.

One of the mechanisms by which the immune system normally acts and regulates itself includes the production of so-called cytokines. It is known that cytokines mediate several positive and negative immune responses. Cytokines normally act by binding to a receptor on a target cell. The activity of cytokines can be interfered with in several ways, for example by administration of soluble receptors (extracellular domains of the receptor) or by cytokine analogues or derivatives.

IL-10 is a cytokine capable of mediating a number of actions or effects. It is known that IL-10 is involved in controlling the immune responses of different classes or subsets of Th cells (T-helper cells).

In US Patent 5,368,854, assigned to Schering Corp., a method is disclosed using IL-10 to treat inflammatory bowel diseases in mammals. In this method the cytokine is administered to a mammal having an IBD (inflammatory bowel disease). The administration of IL-10 as described in this reference is parenteral such as intravascular and preferably intravenous.

It is obvious however that such a route of administration for a (human) patient suffering from an IBD is not without drawbacks. A much easier and more convenient way is an oral administration of a medicament comprising a cytokine such as IL-10 or a cytokine-antagonist which has a similar therapeutic activity. More importantly, localized release of the therapeutic agent allows for higher efficacy and less unwanted side effects due to systemic activities.

In WO 97/14806, assigned to Cambridge University Technical Services Ltd., a method is disclosed for delivering biologically active polypeptides and/or antigens by using non-invasive bacteria, such as *Lactococcus*, by intranasal administration of said polypeptides in the body, especially at the mucosa.

However to treat an inflammatory bowel disease such as chronic colitis or Crohn's disease with a cytokine like IL-10, which is acid sensitive, is a very delicate and difficult task to accomplish. Therefore a system needs to be developed wherein the active compound (e.g. a cytokine or a soluble receptor) is delivered directly at the place where the compound is expected to exert its activity taken into account the problem of acid sensitivity of many cytokines, in particular of IL-10, and the requirement that after oral administration the delivery vehicle needs to pass the acidic environment of the stomach. Furthermore, various digestive enzymes degrade polypeptides as they pass through the stomach and the gut. Last but not least in-situ administration of the agent may allow to reach therapeutically effective concentrations which are difficult to achieve by more systemic routes of administration because of systemic toxicity or other limitations.

In order to achieve the recovery of a patient suffering from an IBD, it is necessary to restore the damaged cells and the organ comprising said damaged cells, for instance the colon.

It is our invention to use a cytokine-producing Gram-positive bacterial strain or a cytokine antagonist producing Gram-positive bacterial strain for the preparation of a medicament to treat inflammatory bowel disease.
Said cytokine or cytokine antagonist to be produced by the bacterial host strain is, for instance, IL-10, a soluble TNF receptor or a cytokine analogue such as the IL-12 derived p40 homodimer (an IL-12 antagonist), an Interferon-γ-antagonist, an IL-1 antagonist or a virus-coded cytokine analogue such as EBV BCRF1 (Baer et al., 1984), whereas the Gram-positive bacterial strain preferably is a *Lactococcus species* and more preferably a *Lactococcus lactis*.
Other Gram-positive bacterial strains to be used for the purpose of the current invention are *Bacillus subtilis*, *Streptococcus gordonii*, *Staphylococcus xylosus*, or a *Lactobacillus spec*.
The inflammatory bowel diseases such as a chronic colitis, Crohn's disease or an ulcerative colitis can be treated according to the invention with an appropriate dosage of the active cytokine compound, preferably IL-10 or soluble TNF receptor, and provides unexpectedly a restoration of the diseased colon to an apparently normal and healthy state.

IL-10 can be administered alone or in combination with at least one additional therapeutic agent. Examples of such agents include corticosteroids, sulphasalazine, derivatives of sulphasalazine, immunosuppresive drugs such as cyclosporin A, mercaptopurine, azathioprine, and another cytokine. The co-administration can be sequential or simultaneous. Co-administration generally means that the multiple (two or more) therapeutics are present in the recipient during a specified time interval. Typically, if a second agent is administered within the half-life of the first agent, the two agents are considered co-administered.

Background information about inflammat ry b wel disease

Inflammatory bowel disease (IBD) refers to a group of gastrointestinal disorders characterized by a chronic non-specific inflammation of portions of the gastrointestinal tract. Ulcerative colitis and Crohn's Disease are the most prominent examples of IBD in humans. They are associated with many symptoms and complications, including growth retardation in children, rectal prolapse, blood in stools (e.g., melena and/or hematochezia), wasting, iron deficiency, and anemia, e.g. iron deficiency anemia and anemia of chronic disease or of chronic inflammation. The etiology or etiologies of IBD are unclear. Reference hereto is made in Wyngaarden and Smith (eds.) *Cecil's Textbook of Medicine* (W.B. Saunders Co. 1985), Berkow (ed.) *The Merck Manual of Diagnosis and Therapy* (Merck Sharp & Dohme Research Laboratories, 1982), and *Harrison's Principles of Internal Medicine*, 12th Ed., McGraw-Hill, Inc. (1991).

Ulcerative colitis refers to a chronic, non-specific, inflammatory, and ulcerative disease having manifestations primarily in the colonic mucosa. It is frequently characterized by bloody diarrhea, abdominal cramps, blood and mucus in the stools, malaise, fever, anemia, anorexia, weight loss, leukocytosis, hypoalbuminemia, and an elevated erythrocyte sedimentation rate (ESR).

Complications can include hemorrhage, toxic colitis, toxic megacolon, occasional rectovaginal fistulas, and an increased risk for the development of colon cancer.

Ulcerative colitis is also associated with complications distant from the colon, such as arthritis, ankylosing spondylitis, sacroileitis, posterior uveitis, erythema nodosum, pyoderma gangrenosum, and episcleritis.

Treatment varies considerably with the severity and duration of the disease. For instance, fluid therapy to prevent dehydration and electrolyte imbalanc is frequently indicated in a severe attack. Additionally, special dietary measures are sometimes useful. Medications include various corticosteroids,

sulphasalazine and some of its derivatives, and possibly immunosuppressive drugs.

Crohn's Disease shares many features in common with ulcerative colitis. Crohn's Disease is distinguishable in that lesions tend to be sharply demarcated from adjacent normal bowel, in contrast to the lesions of ulcerative colitis which are fairly diffuse. Additionally, Crohn's Disease predominately afflicts the ileum (ileitis) and the ileum and colon (ileocolitis). In some cases, the colon alone is diseased (granulomatous colitis) and sometimes the entire small bowel is involved (jejunoileitis). In rare cases, the stomach, duodenum, or esophagus are involved. Lesions include a sarcoid-type epithelioid granuloma in roughly half of the clinical cases. Lesions of Crohn's Disease can be transmural including deep ulceration, edema, and fibrosis, which can lead to obstruction and fistula formation as well as abcess formation. This contrasts with ulcerative colitis which usually yields much shallower lesions, although occasionally the complications of fibrosis, obstruction, fistula formation, and abcesses are seen in ulcerative colitis as well.
Treatment is similar for both diseases and includes steroids, sulphasalazine and its derivatives, and immunosuppressive drugs such as cyclosporin A, mercaptopurine and azathioprine. More recently developed treatments, some still in clinical trials, involve systemic administration (by injection) of TNF blocking compounds such as TNF-antibodies or soluble TNF receptor.
The severe complications of IBD can be seriouly debilitating, and eventually may lead to death.

Some terms used in the current description are, for sake of clarity, explained hereafter.

Generally, the term "symptoms" refers to any subjective evidence of disease or of a patient's condition. This includes evidence as perceived by the patient. Examples of symptoms of IBD include diarrhea, abdominal pain, fever, melena, hematochezia, and weight loss. The term "signs" refers generally to

any objective evidence of a disease or condition, usually as perceived by an examining physician or features which would reveal themselves on a laboratory evaluation or other tests such as an ultrasonic study or a radiographic test. Some examples of signs of IBD include abdominal mass, glossitis, aphtous ulcer, anal fissure, perianal fistula, anemia, malabsorption, and iron deficiency. Occasionally, signs and symptoms overlap. For example, the patient complains of blood stools (a symptom), and a laboratory test of a stool sample is positive for blood (a sign).

The phrase "appropriate dosage" or "effective amount" means an amount or dosage sufficient to ameliorate a symptom or sign of an autoimmune condition or of an undesirable or inappropriate inflammatory or immune response. An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of the side affects.

Detailed description of the methods used in the current invention.

Culture media

GM17 is M17 (Difco, St. Louis) supplemented with 0.5 w/v % of glucose. GM17E is GM17 supplemented with 5µg/ml of erythromycin. BM9 contains per liter 6 g of Na_2HPO_4, 3 g of KH_2PO_4, 1 g of NH_4Cl, 0.5 g of NaCl, 2 mmol of $MgSO_4$, 25 mmol of $NaHCO_3$, 25 mmol of Na_2CO_3, 0.1 mmol of CaCl2, 5 g of glucose and 5 g of casitone (Difco). BM9E is BM9 supplemented with 5µg/ml of erythromycin.

Recombinant DNA techniques.

PCR amplification of DNA was performed with VENT polymerase and using conditions recommended by the manufacturer. DNA modifying enzymes and restriction endonucleases were used under standard conditions and in the buffers recommended by the manufacturers. General molecular cloning techniques and the electrophoresis of DNA and proteins were carried out

essentially as described (Sambrook et al., 1990). *L. lactis* was transformed by electroporation of cells grown in the presence of glycine (Wells et al., 1993).

Construction of the expression plasmids.

The plasmid pT1MIL10 (figure 1) was constructed by subcloning a PCR fragment, obtained with the primers (CAGTACAGCCGGGAAGACAAT and GCACTAGTTAGCTTTTCATTTTGAT) and performed on a cDNA clone containing mIL10 coding sequence. For the design of this strategy we made use of the mIL10 cDNA sequence as given in EMBL acc. nr. M37897. By utilization of the above mentioned primers, the mIL10 fragment could be subcloned as a blunt – SpeI fragment, after treatment with kinase and SpeI, in the NaeI-SpeI opened plasmid pT1NX (figure 1), which is a pTREX1 derivative (Wells and Schofield in : Lactic Acid Bacteria: current advances in metabolism, genetics and applications. F. Bozoglu & R. Bibek, Eds., Nato ASI Series H, Vol.98, p. 37. Springer-Verlag, 1996.)

The plasmid pT1TR5AH (figure 1) was constructed by subcloning a PCR fragment, obtained with the primers (CTGGTCCCTTCTCTTGGTGAC and CCACTAGTCTATTAATGATGATGATGATGATGCGCAGTACCTGAGTCCTG GGG) and performed on a cDNA clone containing sTNFr55 coding sequence. For the design of this strategy we made use of the TNFr55 cDNA sequence as given in EMBL acc. nr. L26349. By utilizing the above mentioned primers, the sTNFr 55 fragment was provided with a 6his tag at the 3'end and could be subcloned as a blunt – SpeI fragment, after treatment with kinase and SpeI, in the NaeI-SpeI opened plasmid pT1NX.

Both plasmids code, downstream from the lactococcal P1 promotor, for fusion genes between the secretion leader from Usp45 (Van Asseldonk et al., Gene, 95, 155-160,1990) and mIL10 and sTNFr 55, respectively. Upon secretion, the leader sequence is cleaved off.

Id ntification of recombinant proteins

Recombinant mIL10 and msTNFr 55 could be observed in the supernatant of cultures of MG1363[pT1MIL10] and MG1363[pT1TR5AH], respectively (figure

2). For this test, 5 ml aliquots of the cultures were extracted with 2 ml phenol and the proteins were subsequently prepared from the organic phase by precipitation with 10 ml of ethanol. A part of the precipitate, equivalent to 1 ml of culture supernatant, was subjected to SDS-15% PAGE and immunoblotting. Culture samples were taken at relevant times in the growth phase of the bacteria, as described below.

The culture supernatant of MG1363[pT1MIL10] contained, on average, 1 $\mu g.ml^{-1}$ of murine IL10. Murine IL-10 activity of the supernatant was measured using a murine mast cell line MC/9 (Thompson-Snipes, L. et al., J. Exp. Med. 173, 507, 1991). Human IL-10 binds to murine IL-10R as was demonstrated by transfection experiments (Ho, A.S.Y et al., PNAS 90, 11267, 1993; Liu, Y. et al., J.Immunol. 152, 1821, 1994). 1 $U.ml^{-1}$ of IL-10 is defined as the amount of IL-10 that is able to inhibit 50% the level of IFN-gamma production of conA activated splenocytes (Fiorentino, D.F. et al., J.Exp.Med. 170, 2081, 1989). The ED50 for this effect is typically 0.3-0.6 $ng.ml^{-1}$. When measured along with a standard of known activity (Biosource International, CA) the MG1363[pT1MIL10] culture supernatant revealed an activity of approximately 8000 $U.ml^{-1}$. Berg et al. (J. Clin. Invest 98, 1010-1020) report a specific activity of approximately 1.0×10^7 $U.mg^{-1}$ for recombinant mIL10. From these considerations and taking into account the variations in the method used, we concluded that the recombinant mIL10, present in the MG1363[pT1MIL10] culture supernatant, displayed full biological activity. No IL10 activity could be detected in the supernatant of the control cultures, MG1363 or MG1363[pTREX1].

The culture supernatant of strain MG1363[pT1TR5AH] contained, on average, 200 $ng.ml^{-1}$ msTNFr 55. Loetscher et al. (1991) showed that complete inhibition of TNF cytotoxic activity by sTNFr 55 was only obtained from a molar ratio of 1000 : 1 of sTNFr 55 to TNF and higher. The soluble recombinant TNFr 55 which had been recovered from the culture supernatant of MG1363(pT1TR5AH) showed an equal inhibitory effect on TNF as had been reported for the indigenous product. This was demonstrated by mixing up and thus competing out a titration series of TNF with a titration series of

recombinant sTNFr and measuring TNF activity in a cytotoxicity assay as described (Espevik, T and Nissen-Meyer, 1986).

Pretreatment of the mice

For the induction of chronic colitis, mice were pre-treated as described by Kojouharoff et al. Clin Exp Immunol 107, 353, 1997. Six to eight weeks old female Balb/c mice received four cycles of treatment with DSS. Each cycle consisted of 5% DSS in the drinking water for 7 days, followed by a 10-day interval during which they received normal drinking water. Four to six weeks after completion of the last DSS cycle, mice were treated with the *L. lactis* strains as indicated.

In order to further disclose and thus clarify the current invention some examples are given hereunder.

Examples

Example 1.

Treatment of the mice with live *L. lactis*

Storage of expression strains

Freshly streaked cultures of the *L. lactis* expression strains were inoculated in 10 ml of GM17 or GM17E depending on the absence or presence of an expression plasmid and grown overnight at 30°C. The overnight cultures were diluted 1/100 in fresh GM17 or GM17E and pregrown for 3 hours at 30°C. The cells were harvested by centrifugation and resuspended in BGM9 or BGM9E, depending on the presence of plasmids. These cultures were grown for 5 hours at 30°C. The protein profile of these cultures was analysed by performing Western immunoblotting on an equivalent of 1 ml of culture supernatant using either antiserum directed towards sTNFr 55 or IL10 respectively. The protein profile showed the presence of sTNFr 55 and IL10 in the appropriate lanes (figure 2). 5 ml of the original GM17 or GM17E overnight cultures was supplemented with 5 ml of glycerol and stored at –20°C. These stocks were used as starter material for several experiments. Protein analysis throughout a series of individual experiments showed that a high degree of reproducibility in the production of the recombinant proteins could be obtained by this procedure.

Weeks 1 and 2

Stock solutions of *L. lactis* strains were diluted 1/200 in 10 ml GM17 or GM17E and grown overnight at 30°C. The cells were harvested by centrifugation and resuspended in 1 ml BM9 or BM9E. Control, healthy mice and mice with induced colitis were inoculated on a daily basis with 100 µl aliquots of these cell suspensions.

Weeks 3 and 4

Stock solutions of *L. lactis* strains were diluted 1/200 in 10 ml GM17 or GM17E and grown overnight at 30°C. These cultures were diluted 1/25 in 10 ml of BM9 or BM9E and grown for 3 hours at 30°C. Aliquots of 200 µl were intragastrically administered into mice on a daily basis.

Example 2.

Determination of histological score

Histological score was determined essentially as described by Kojouharoff et al. Clin Exp Immunol 107, 353, 1997.

Mice were killed by cervical dislocation. The colon was removed and washed with PBS. The distal third of the colon was cut longitudinally, laid on filter paper and fixed with 10% formalin in PBS overnight. Sections of the parafin-embedded material were made longitudinally. Three 3-µm sections were cut at an intermediate distance of 200 µm. The sections were stained with haematoxylin-eosin. Histological analysis was performed in blind fashion. Mice were scored individually, and each score represented the mean of three sections.

Histology was scored as follows:

Infiltration : 0, no infiltrate; 1, infiltrate around crypt bases; 2, Infiltrate reaching to L. muscularis mucosae; 3, extensive infiltration reaching the L. muscularis mucosae and thickening of the mucosa with abundant oedema; 4 infiltration of the L. submucosa.

Epithelial damage: 0, normal morphology; 1, loss of goblet cells; 2, loss of goblet cells in large areas; 3, loss of crypts; 4, loss of crypts in large areas and/or foci of polyploid regeneration.

Colonic length was measured immediately after dissection and placement on a paper towel.

The pathology of chronic colitis is, amongst other parameters, characterised by a decrease in length of the colon and by epithelial damage and infiltration of lymphocytes to a more or less substantial extent.

Figure 3 clearly shows an increase in colon length after the treatment of the inflamed mice with MG1363[pT1MIL10] and, although to a lesser extent, after the treatment of the mice with MG1363[pT1TR5AH].

Figure 4 and 5 show the onset of recovery from chronic colitis, in which mice treated with MG1363(pT1MIL10) appear to improve more extensively than those mice which had been treated with MG1363[pT1TR5AH].

Figure 4 shows the histological score of epithelial damage whereas figure 5 shows inflammatory infiltrate, both determined as described previously.

Figure 6 shows the histology of normal tissue, compared to inflamed and treated tissue.

In the normal histology one can observe a continuous array of crypts of equal length. In the crypts, numerous goblet cells can be observed. A low number of lymphocytes is present in the mucosa. No lymphocytes are present in the submucosa. In the inflamed tissue, one can see the disappearance of the organised crypt structures, ranging from differences in length to complete absence of structure. Also, in the relicts of the crypts no goblet cells are present. One can observe a large increase of the thickness of the mucosa due to a massive infiltration of lymphocytes. The lymphocytes tend to form ulcerations. In severe cases, infiltration of lymphocytes can also be observed in the submucosa. The epithelium, however, remains intact. The negative control of treatment with MG1363(pTREX1) shows a pathology reminescent of that of heavilly inflamed tissue. Mice treated with MG1363 (pT1MIL10) show an almost complete restitution of the normal histology, revealing only slight remainders of infiltrating lymphocytes in the mucosa. Mice treated with MG1363[pT1TR5AH] show an intermediate degree in pathology.

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Claims

1. Use of a cytokine-producing Gram-positive bacterial strain or a cytokine antagonist-producing Gram-positive bacterial strain for the preparation of a medicament to treat inflammatory bowel disease.
2. Use of a Gram-positive bacterial strain according to claim 1 wherein the cytokine or cytokine antagonist is IL-10, a soluble TNF receptor or another TNF antagonist, an IL-12 antagonist, an Interferon-γ antagonist, an IL-1 antagonist or a virus-coded cytokine analogue such as EBV BCRF1.
3. Use of a Gram-positive bacterial strain according to claim 1 or 2 wherein the Gram-positive bacterial strain is a *Lactococcus species*.
4. Use of a Gram-positive bacterial strain according to claim 3 wherein the *Lactococcus species* is *Lactococcus lactis*.
5. Use of a Gram-positive bacterial strain according to claim 1 or 2 wherein the Gram-positive bacterial strain is *Bacillus subtilis*, *Streptococcus gordonii*, *Staphylococcus xylosus*, or a *Lactobacillus spec*.
6. Use of a Gram-positive bacterial strain according to any of the preceeding claims wherein the bowel disease is a chronic colitis, Crohn's disease or an ulcerative colitis.

Abstract

The current invention relates to an administraton strategy for the delivery at the intestinal mucosa of cytokines or cytokine antagonists, preferably of acid sensitive anti-inflammatory agents, such as IL10 and/or soluble TNF receptor via the oral route. The prefered feature according to the invention is the inoculation with a suspension of recombinant *Lactococcus lactis* cells, which had been engineered to produce the respective proteins.

Legends to figures

Figure 1 :
Overview of the plasmids used
Figure 1a : schematic maps of the plasmids used. P1 is the lactococcal P1 promotor as in Waterfield et al, (1995), usp45S is a DNA fragment encoding the secretion signal peptide from the lactococcal Usp45 (van Asseldonck et al, 1990), mil10 is a DNA fragment encoding the mature part of murine interleukin 10, tr55 is a DNA fragment encoding the soluble part of type 1 TNF receptor, H6 is a fragment encoding 6 histidine residues, Em^r is the erythromycin selection marker.
Figure 1b : DNA sequences of pTREX1 and pT1NX
Figure 1c : DNA sequences of pT1MIL10 and pT1TR5AH

Figure 2 :
Protein profile following SDS-PAGE of the culture supernatant of the indicated strains after immunoblot, revealed with anti-murine interleukin 10 (panel A) or anti-murine type 1 TNF receptor and anti-6 His (panel B) antisera.

Figure 3 :
Average of colon length of groups of mice in which : a) chronic colitis had been induced with DSS, b) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pTREX1 was orally administered, c) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1TR5AH was orally administered and d) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1MIL10 was orally administered.

Figure 4 :
Average of epithelial damage score in the distal colon of groups of mice in which : a) chronic colitis had been induced with DSS, b) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pTREX1 was orally administered, c) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1TR5AH was orally administered and d) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1MIL10 was orally administered.

Figure 5 :
Average of inflamatory infiltrate score in the distal colon of groups of mice in which : a) chronic colitis had been induced with DSS, b) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pTREX1 was orally administered, c) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1TR5AH was orally administered and d) chronic colitis had been induced with DSS and to which subsequently *L. lactis* strain MG1363pT1MIL10 was orally administered.

Figure 6 :
Representative sections of mice distal colon stained with haematoxylin and eosin.
normal tissue : untreated animals
DSS colitis : animals pretreated with DSS to acquire chronic colitis
DSS colitis, MG1363pT1MIL10 treatment : animals pretreated with DSS to acquire chronic colitis to which subsequently *L. lactis* strain MG1363pT1MIL10 was orally administered.
DSS colitis, MG1363pTREX1 treatment : animals pretreated with DSS to acquire chronic colitis to which subsequently *L. lactis* strain MG1363pTREX1 was orally administered.

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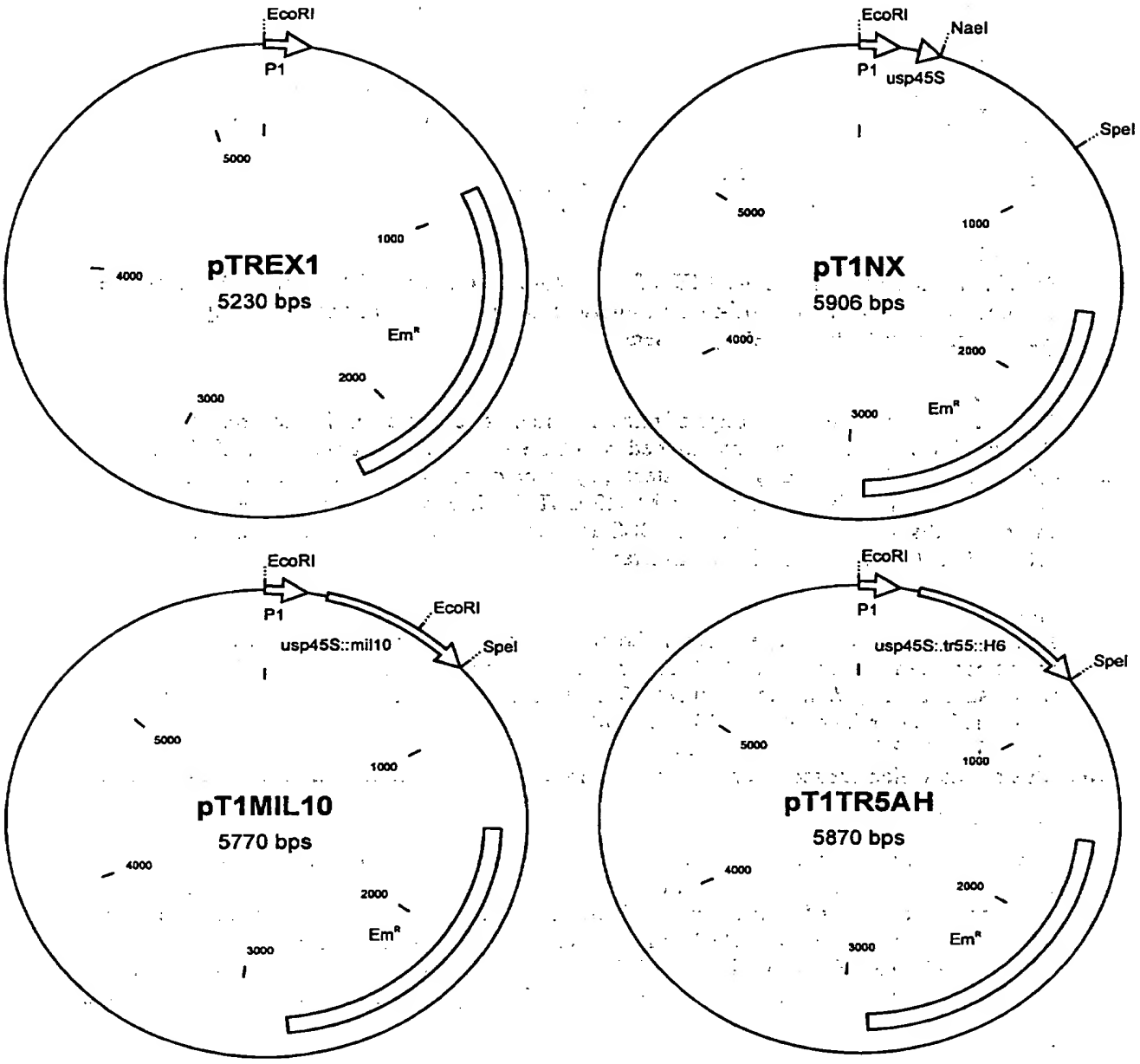

Figure 1a

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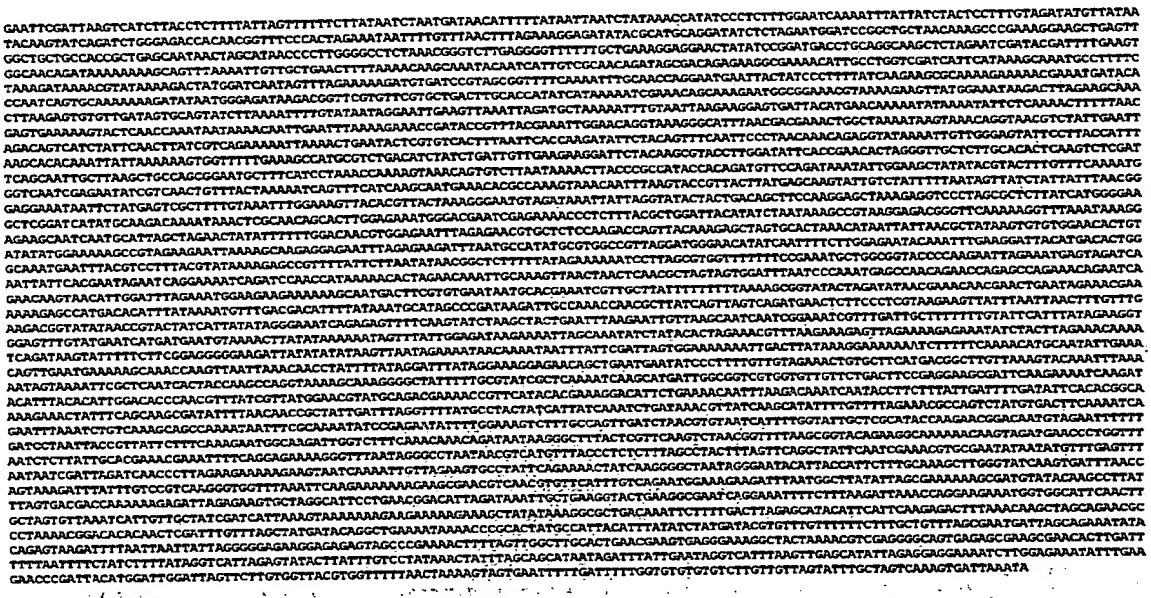

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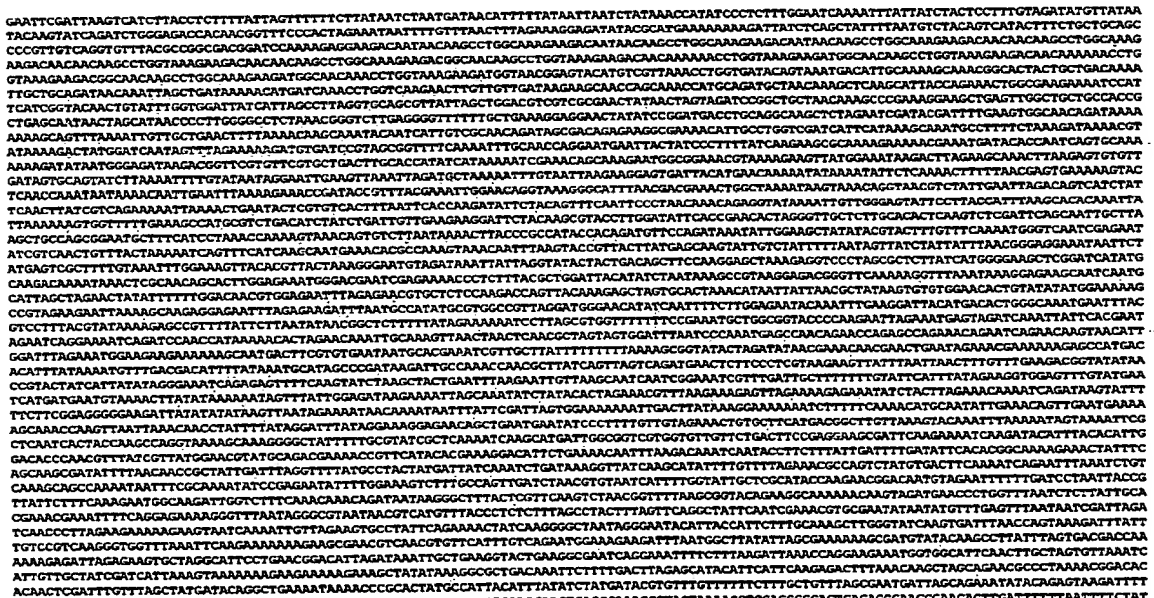

Figure 1b

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pT1TR5AH

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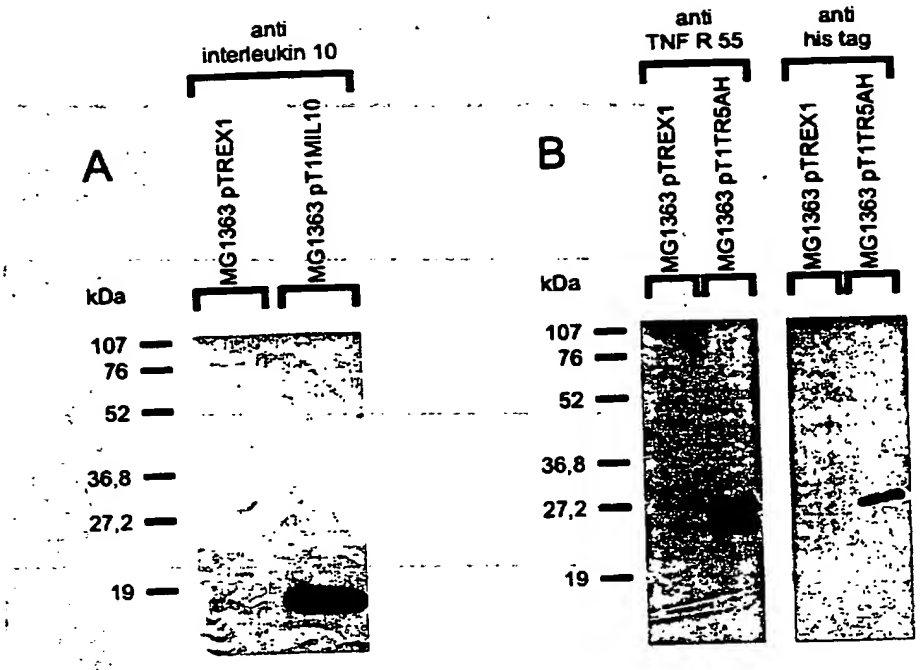
Figure 1c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his tag
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76
52
36,8
27,2
19

Figure 2

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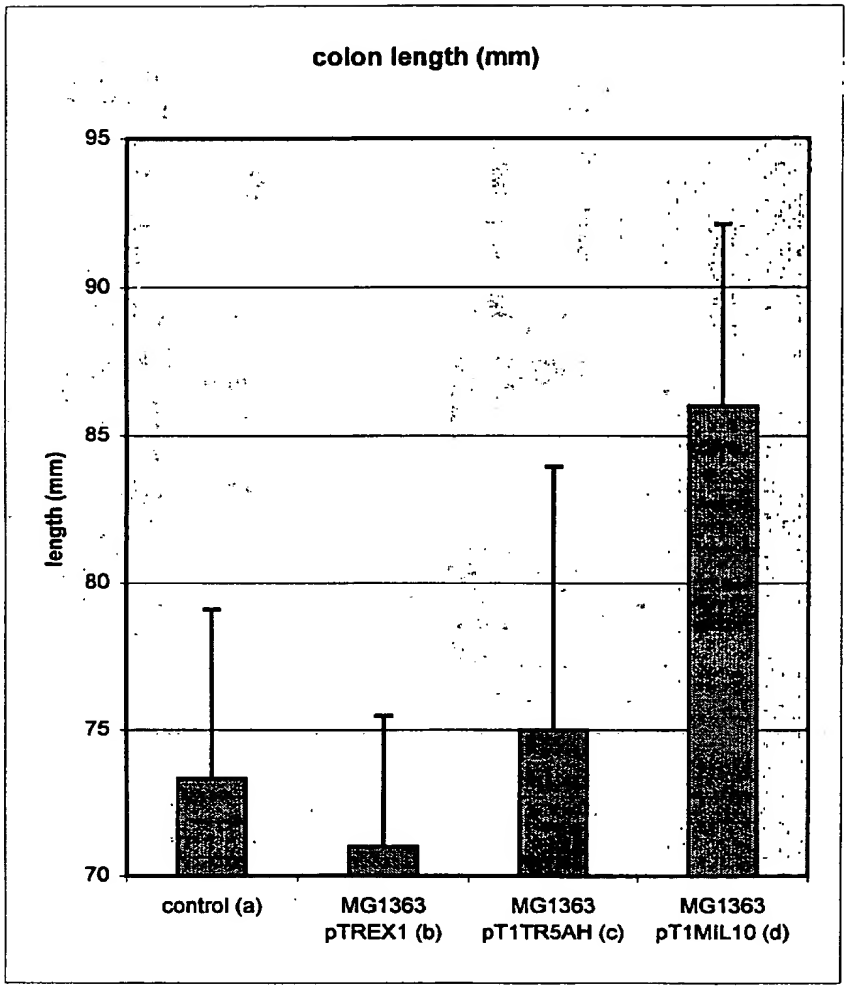

Figure 3

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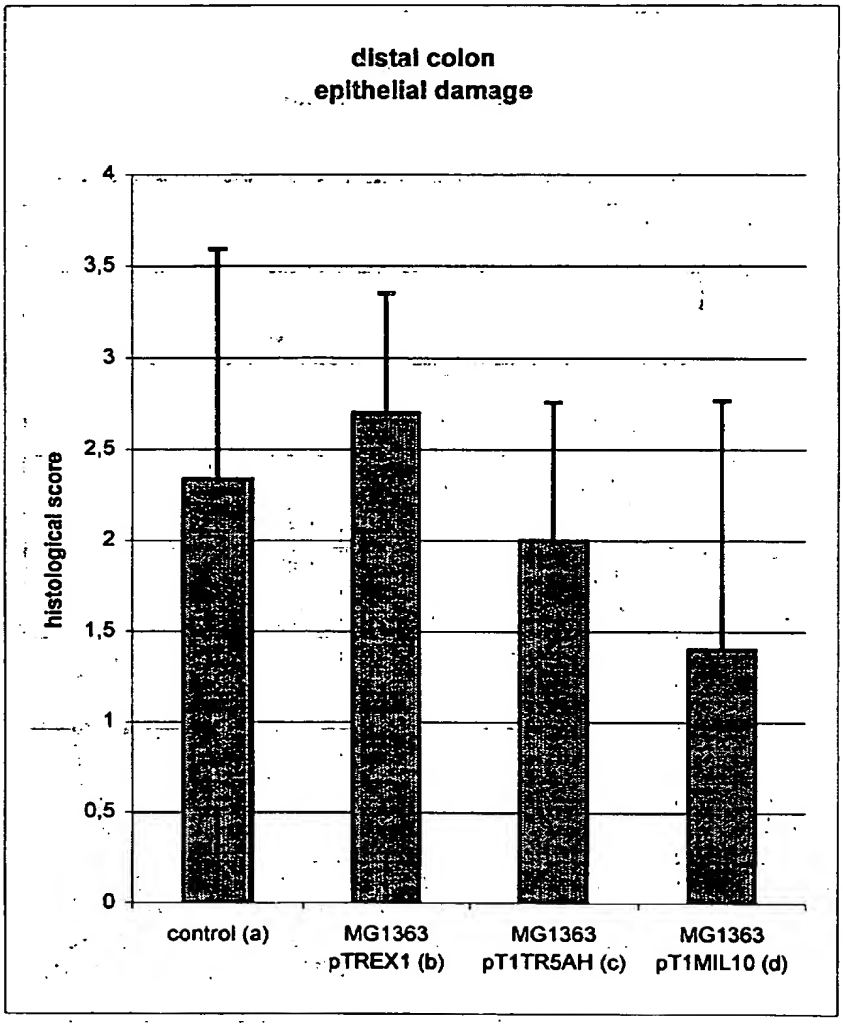

Figure 4

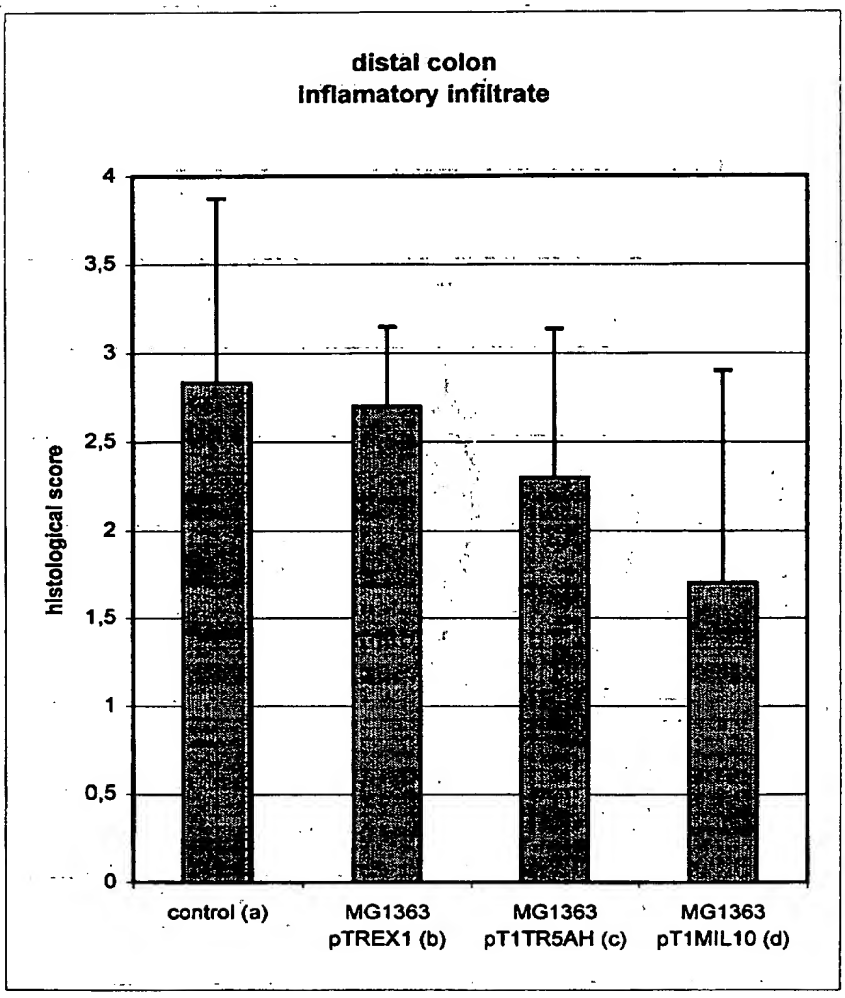

Figure 5

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Normal tissue

DSS colitis, MG1363 pT1MIL10 treatment

DSS colitis

DSS colitis, MG1363 pTREX1 treatment

Figure 6